Cytogenetics and genetic engineering

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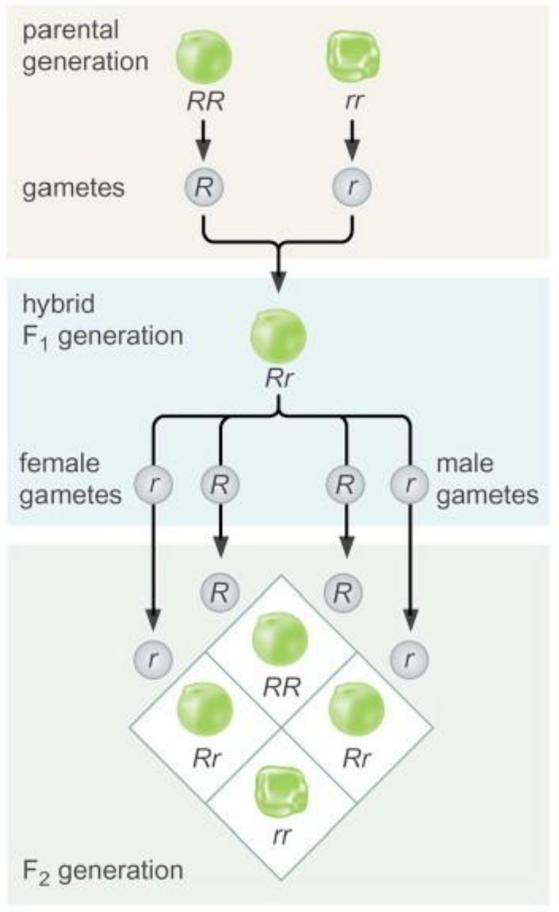
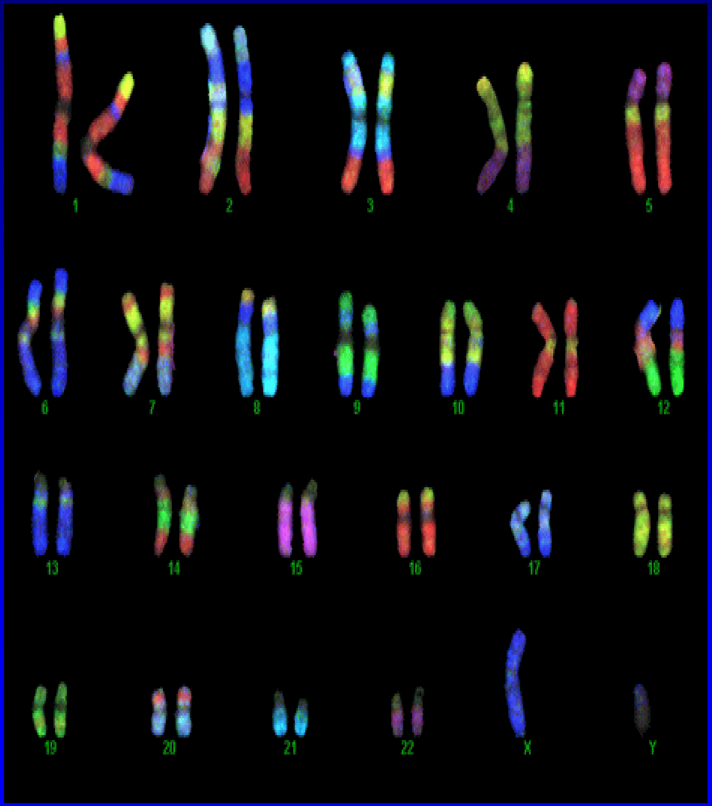
History: Hereditary Traits are Passed to Progeny



- In 1865 Mendel presented work demonstrating that hereditary traits are passed onto progeny.
- Followed simple characteristics of strains of peas (round vs. wrinkled; yellow vs. green).
- Crosses of peas with different characteristics demonstrated that some traits were present in the progeny (**dominant trait**) and some were not (**recessive trait**) and that these traits appeared in a certain ratio (dominant - 75%; recessive - 25%).
- Concluded - the traits are controlled by pairs of factors (now known to be **genes**). One factor derives from the mother and one from the father.
- The resulting progeny contains two versions (or **alleles**) of the trait and these alleles (dominant or recessive) determine the appearance of the trait of the progeny.

History: Hereditary Traits are Passed to Progeny



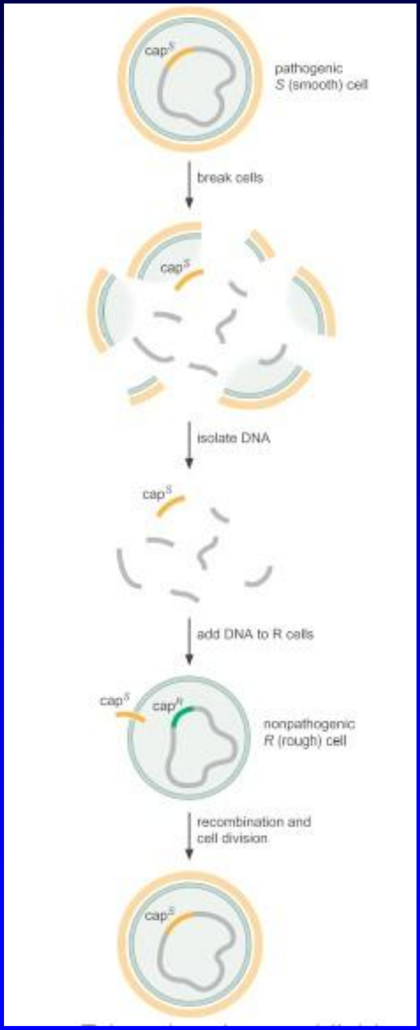
Phenotype - the appearance or physical structure of an individual (round vs. wrinkled; red vs. pink vs. white)

Genotype - the genetic composition of an individual

Homozygous - a gene pair in which both the maternal and paternal genes are identical (AA or aa)

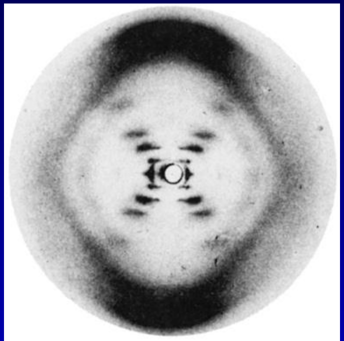
Heterozygous - a gene pair in which the maternal and paternal genes are different (Aa or aA)

History: DNA Carries Genetic Specificity



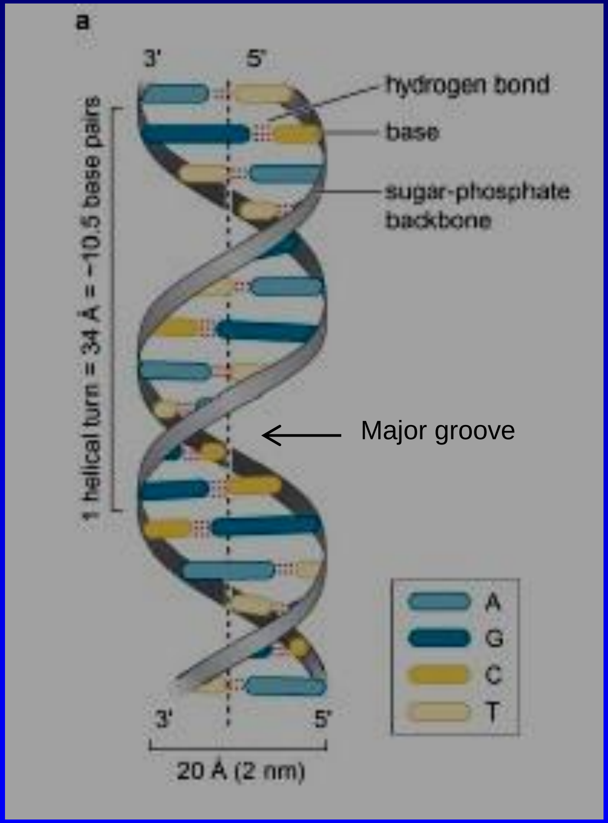
- **Frederick Griffith** demonstrated in 1928 that non-virulent strains of bacteria become virulent when mixed with their heat-killed pathogenic counterparts.
- Indicated that the genetic components are undamaged by heat and that these components can pass through the cell wall of the living recipient and recombine (or insert itself) into the new host.
- In 1944, **Oswald T. Avery** and his colleagues demonstrated that DNA was the active genetic component.
- They broke open pathogenic cells, isolated the DNA and introduced the pathogenic DNA to non-pathogenic cells.
- Resulting bacteria became pathogenic and this process could be destroyed by treating the DNA with a recently purified enzyme pancreatic deoxyribonuclease (degrades DNA) but not a ribonuclease (degrades RNA) or proteolytic enzymes (degrades protein).

History: The Revolution of the Double Helix



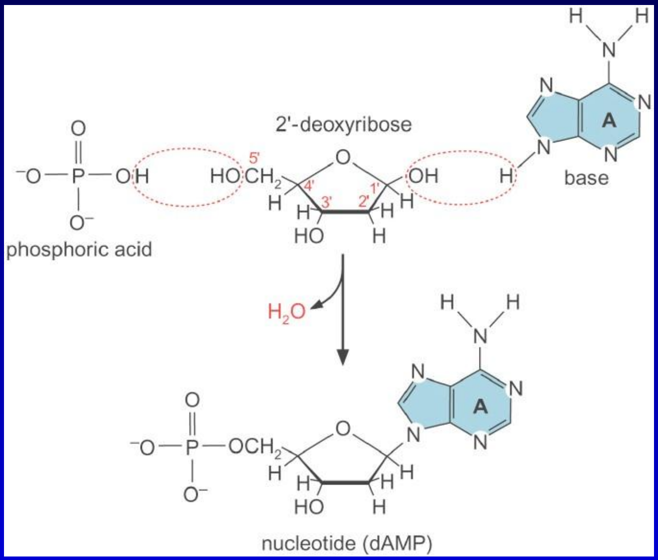
- In 1952, **Rosalind Franklin** took the first high-quality X-ray diffraction pictures of DNA. (Rosalind Franklin: The Dark Lady of DNA).
- In 1953, **Francis Crick** and **James Watson** used (more accurately stole) this picture to determine that DNA exists in a complementary double helix.
- In this helix, the two strands are held together by weak chemical bonds between base pairs on opposing strands.
- This pairing occurs in a very specific manner (A:T/G:C).
- The solution of the structure of DNA revolutionized the manner in which scientists studied DNA and molecular biology.
- DNA was now a real molecule that could be studied and thought about objectively.
- The complementary strands suggested that one strand served as the template from which a second strand was synthesized.
- If true, (which it was), the fundamental problem initiated by Mendel’s observations would be solved.

DNA structure



- DNA is composed of two **<u>polynucleotide chains</u>** twisted around each other to form a double helix.
- The helix is usually, but not always, right handed.
- It contains nucleotide bases hydrogen bonded to each other and a sugar-phosphate backbone (linking bases at the DNA single strand).
- There are 10.5 base pairs for each turn of the helix.
- The nature of the nucleotides in the alpha helix create a major groove and a minor groove in which the bases themselves are accessible to solution.
- Major and minor groove are determined based on the distance between the phosphate backbone

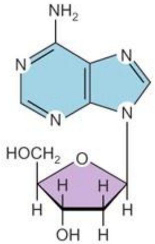
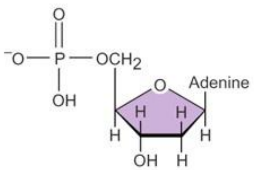
The Nature of Nucleotides



- The DNA nucleotide contains a **2'-deoxyribose** sugar backbone (called such because of the lack of a hydroxyl group at the 2' position).
- A phosphate group is attached to the sugar at the 5'-OH position.
- A base is attached to the sugar at the 1'-OH position.

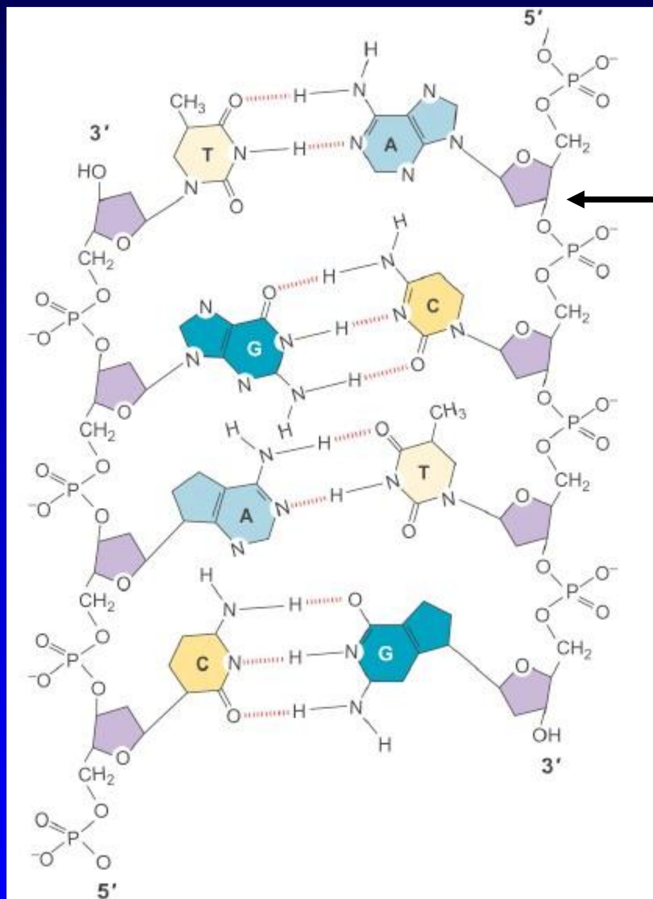
Notice the difference in naming (base, nucleoside, nucleotide), which depends on the presence of the base alone, the base attached to the sugar, or the base and the phosphate attached to the sugar.

TABLE 6-1 Adenine and Related Compounds

	Base Adenine	Nucleoside 2′-deoxyadenosine	Nucleotide 2′-deoxyadenosine 5′-phosphate
Structure			
Molecular weight	135.1	251.2	331.2

The Formation of the DNA Chain

5' to 3' antisense strand



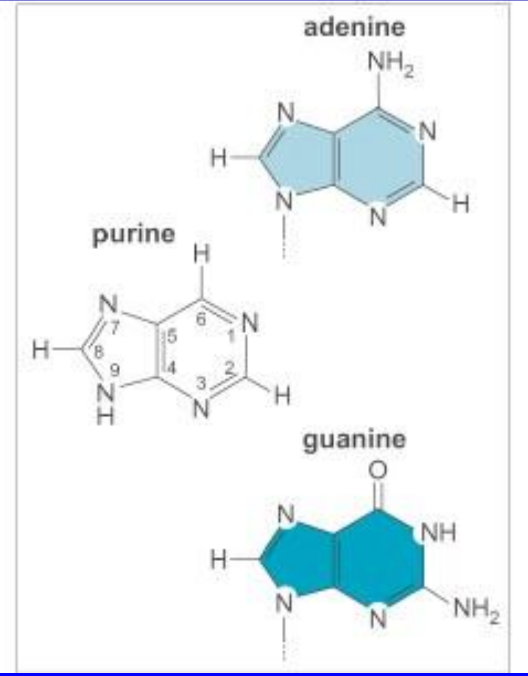
5' to 3' (sense strand)

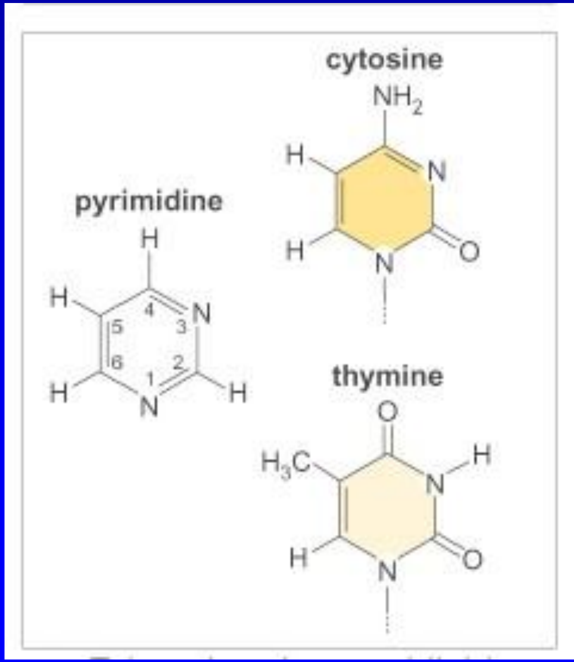
- The nucleotides are then joined to each other into a polynucleotide chain.
- The 5'-phosphate of one nucleotide is esterified to the 3'-OH of the second nucleotide.
- This forms a **phosphodiester linkage** to create the repeating sugar-phosphate backbone of the DNA strand.
- This formation gives a "polarity" to the DNA - DNA chains have a free 5'-phosphate at one end and a free 3'-OH at the other end (5' to 3').
- Convention is to read DNA from the 5' to the 3' end and the strands are said to have an **antiparallel orientation**.

The Bases of DNA - Structure

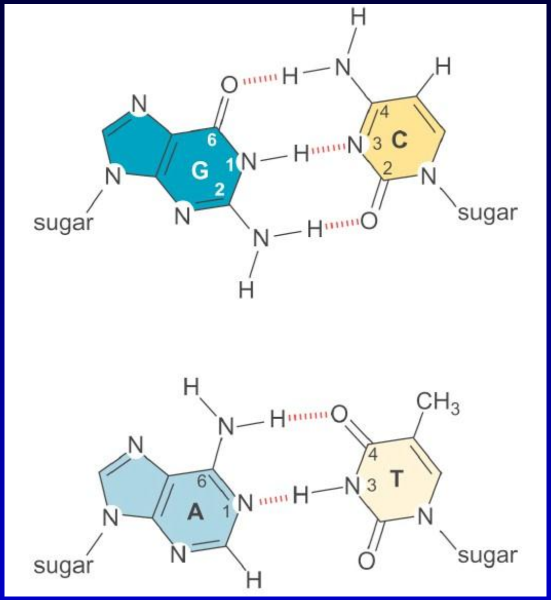
- The DNA bases fall into two categories:
  - **Purines** - adenine and guanine
  - **Pyrimidines** - cytosine and thymine
  - (When you CUT a Py you get a GA-P)

NOTE: KNOW THESE STRUCTURES!!!!!!





The Bases of DNA - Base Pairing

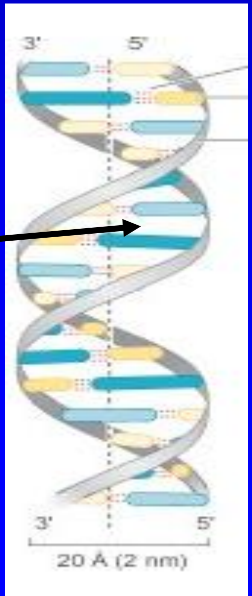


- The two polynucleotide chains are held together by hydrogen bonding between the bases of the nucleotides on complementary strands.
- Adenine ALWAYS pairs with Thymine in DNA
- Guanine ALWAYS pairs with Cytosine in DNA

<u>NOTE: KNOW THESE BASE PAIRINGS!!!!!!!!</u>

- This base pairing gives a complementary relationship between the two strands of DNA and gives the specificity of the base pairing:
  - 5'-ATGTC-3' on the **<u>sense</u>** strand will always have 3'-TACAG-5' on the **<u>antisense</u>** strand.

Base stacking and π - π interactions.



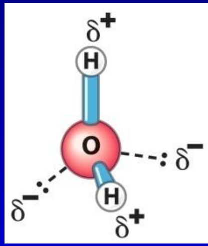
- The stability of the double helix comes from several thermodynamic principles:
  - Base pairing disrupts water interactions with the bases increasing entropy (hydrophobic interactions)
  - The extensive hydrogen bonding of the DNA chain.
  - Base stacking allows electron cloud interactions between nucleotides in the chain (π- π). (Similar to Van der Waals interactions.)

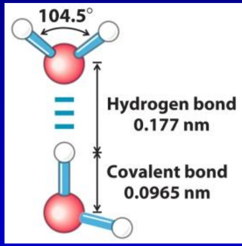
The Chemical Forces Involved in Molecular Biological Mechanisms

Weak Chemical Bonds in Molecular Biology

- The proteins and nucleotides that make up the molecules important in Molecular Biology are joined and combined by different types of chemical bonding.
- A **chemical bond** is an attractive force that holds atoms together and are of primarily two types:
  - **Covalent Bonds** - strong, stable bonds that essentially never break under physiological conditions. These are used to join amino acids together to make proteins and peptides or to join nucleotides together to make RNA and DNA. (phosphodiester bond)
  - **Weak Bonds** - these are low energy bonds that can be easily broken under physiological conditions (ionic, hydrogen, Van der Waals, and hydrophobic). These are used to mediate the interactions between proteins and DNA/RNA, between proteins, within proteins, or within DNA (i.e. - to form the double helix) and serve as the basis for how biomolecules interact to “carry out” the central dogma of molecular biology.

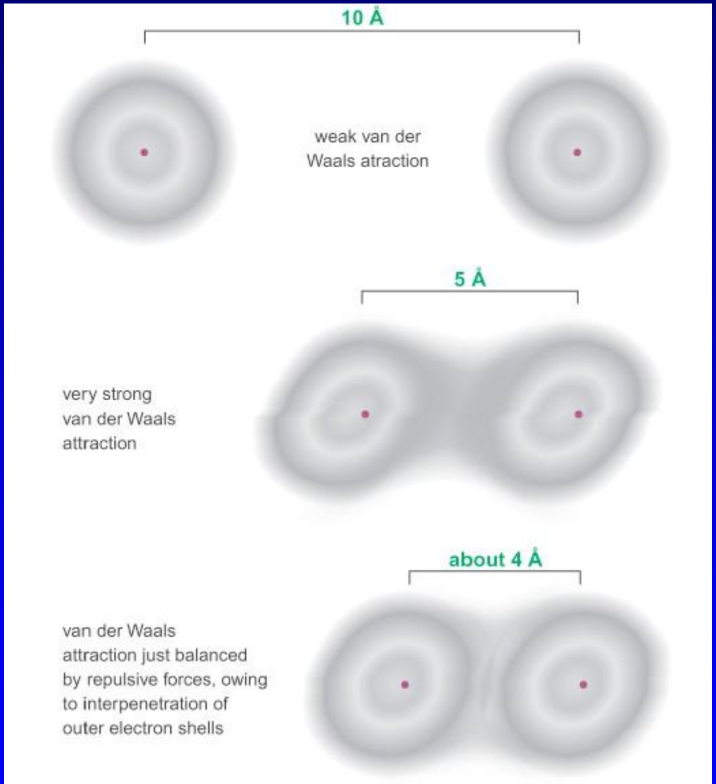
Weak Chemical Bonds: Hydrogen Bonding





- Water as an example - the Oxygen (O) nucleus attracts electrons more strongly than does the hydrogen (H) nucleus. Therefore, O is more electronegative than H.
- Because of the difference in electronegativity, the sharing of electrons is unequal, which results in an electric dipole (i.e. - the H atom contains a partial positive charge and the O atom contains a partial negative charge.)
- A hydrogen bond is formed between a covalently bound donor hydrogen atom with partial positive charge (due to the polarity of the molecule) and an acceptor atom (N and O most commonly) that contains a partial negative charge.
- Common hydrogen donors:
    - N-**H** (in -NH_2) and O-**H** (in an hydroxyl group).
- Common hydrogen acceptors:
    - C=**O** (in -CO_2) and **N** (in peptide bonds and between the two strands of DNA, etc.)

Weak Chemical Bonds: Van der Waals Forces



- These interactions arise because the electron clouds of two non-charged atoms brought in close proximity will influence each other.
- This influence produces random variations in the electrons, inducing a transient, opposite electric dipole in the nearby atom.
- The result is a weak attraction between the two non-charged atoms.
- This occurs between all molecules (nonpolar and polar) and depends on the distance between the interacting molecules.
- The radius of the interaction is dependent on the Van der Waals radius for each molecule, which is the point at which the attractive and repulsive forces balance out.

Weak Chemical Bonds: Ionic and Hydrophobic

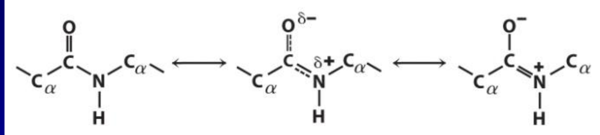
Ionic Interactions: a simple attraction between two species of opposite charge.

DNA
|
Lys - NH_3^+ ⟶ ⟵ $^-$O - P - DNA
||
O

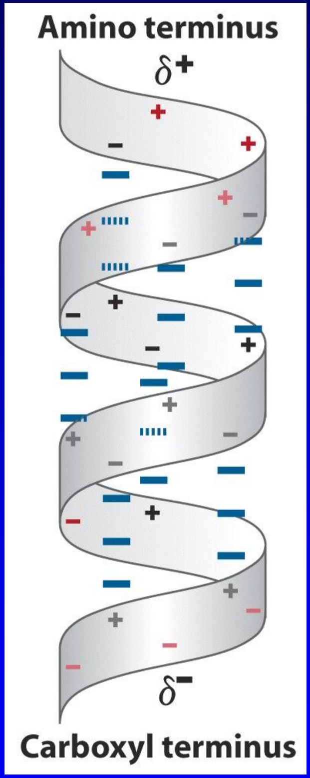
Hydrophobic interactions: The strong tendency of water to exclude nonpolar groups to create the greatest thermodynamic stability within a molecule. Nonpolar groups will try to arrange themselves so that they are not in contact with water (like surrounding like). These interactions are important in the stabilization of proteins, the interactions between proteins, or the partitioning of proteins into a membrane layer (not to be covered in this course). (THINK: OIL AND WATER…. THEY DON'T MIX.)

Weak Chemical Bonds: Helical Dipole

Helical Dipole:



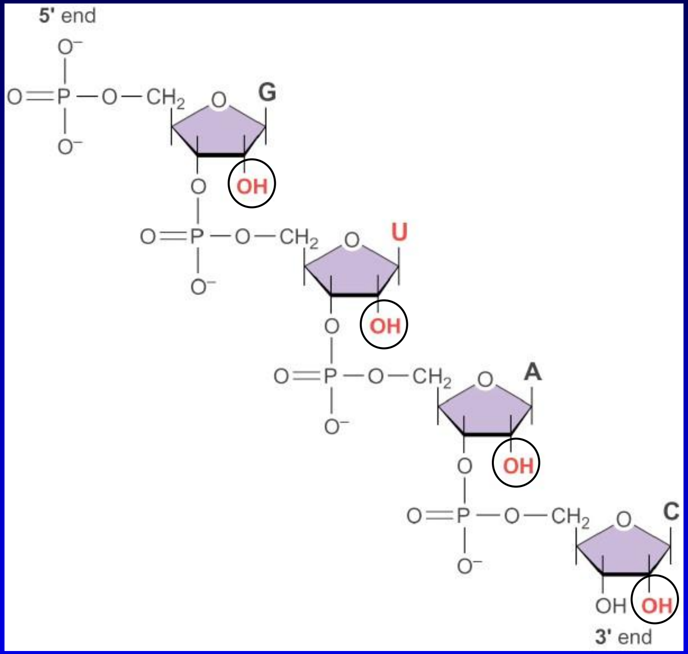
The carbonyl oxygen has a partial negative charge and the amide nitrogen a partial positive charge, setting up a small electric dipole.



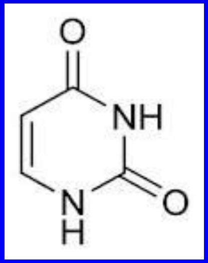
The partial negative and positive charges on the peptide carbonyl and amide nitrogen are connected through the helix through the network of H-bonding. This connection results in a net dipole throughout the helix with a net positive charge on the amino terminus and a net negative charge on the carboxyl terminus.

This net dipole creates a situation where a “large scale” ionic interaction can occur between the net positive amino terminus and the negative phosphate backbone.

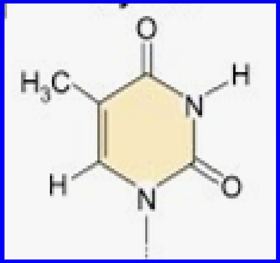
RNA Structure



Uracil



Thyamine



RNA like DNA, is comprised of a string of nucleotides joined by covalent sugar-phosphate backbone bonds. RNA is similar to DNA except in three important ways:

1) It utilizes a ribose ring instead of a deoxyribose ring. That is, it contains a hydroxyl group at the 2' position instead of a hydrogen.

2) RNA contains uracil instead of thymine. Uracil is the identical structure to Thymine EXCEPT it is lacking a methyl group at the 5' position. Like thymine, uracil predominantly pairs with adenine.

3) RNA is usually found as a single polynucleotide chain and not as an extensive double helix.

Unlike DNA, RNA is not the genetic material and as such has no need to be able to replicate. Instead, it acts as the messenger between DNA and protein (mRNA), an adaptor to assist in protein synthesis (tRNA), a regulator to inhibit protein synthesis (siRNA), and in some instances as enzymes (ribosomal RNA and ribozymes).

END